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Measurement of unbound ranitidine in blood and bile of anesthetized rats using microdialysis coupled to liquid chromatography and its pharmacokinetic application

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Abstract

To investigate the pharmacokinetics of unbound ranitidine in rat blood and bile, multiple microdialysis probes coupled to a liquid chromatographic system were developed. This study design was parallel in the following groups: the control-group of six rats received ranitidine alone (10 and 30 mg/kg, i.v.), the treated-group rats were co-administered with ranitidine and cyclosporine (*P*-glycoprotein (*P*-gp) inhibitor) or quinidine (both organic cation transport (OCT) and *P*-gp inhibitors) in six individual rats. Microdialysis probes were inserted into the jugular vein and the bile duct for blood and bile fluids sampling, respectively. Ranitidine in the dialysate was separated by a reversed-phase C_{18} column (Zorbax, 150 mm × 4.6 mm i.d.; 5 μm) maintained at ambient temperature. Samples were eluted with a mobile phase containing acetonitrile–methanol–tetrahydrofuran–20 mM K_2HPO_4 (pH 7.0) (24:20:10:946, v/v), and the flow rate of the mobile phase was 1 ml/min. The optimal UV detection for ranitidine was set at wavelength 315 nm. Between 20 and 30 min after drug administration (10 or 30 mg/kg), the ranitidine reached the maximum concentration in the bile. The bile-to-blood distribution ratio (AUC_{bile}/AUC_{blood}) was 9.8 ± 1.9 and 13.9 ± 3.8 at the dosages of 10 and 30 mg/kg, respectively. These studies indicate that ranitidine undergoes hepatobiliary excretion which against concentration gradient from bile-to-blood. In addition, the AUC of ranitidine in bile decreased in the treatment of cyclosporine or quinidine, which suggests that the hepatobiliary excretion of ranitidine was partially regulated by *P*-glycoprotein or organic cation transporter.

Keywords: Hepatobiliary excretion; Microdialysis; *P*-glycoprotein; Pharmacokinetics ranitidine

1. Introduction

Ranitidine {*N*-(3-[{[5-(dimethylamine)methyl-2-furanyl] methyl} thio]ethyl) - N9 - methyl -2-nitro-1,1-ethenediamine hydrochloride} is the H_2-receptor antagonist used for peptic ulcer [1]. Analysis of ranitidine from biological samples is commonly performed using high-performance liquid chromatography (HPLC) coupled to UV detection [2,3] and tandem mass spectrometry [4,5]. Sample preparation of ranitidine from human plasma has been accomplished by deproteination using perchloric acid [3] and solid-phase extraction [5,6]. On the whole, these methods are time-consuming, and require tedious procedures for the preparation of biological samples. However, only protein-unbound drugs are available for drug distribution to the target site and for therapeutic application. To date, measurement of protein-unbound ranitidine in the blood and bile has not been described. Microdialysis technique provides an in vivo method to monitor unbound drug in various biological fluids, which excludes large molecule out of the dialysis membrane [7].

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Since ranitidine interacts with the *P*-glycoprotein (*P*-gp), clarification of the transport mechanism may provide important information for studying the pharmacokinetics of ranitidine. It is also to be noted that not all *P*-glycoprotein substrates are subject to significant biliary excretion. Thus, to obtain more detailed information about the disposition of ranitidine in vivo, this study investigates the pharmacokinetics of unbound ranitidine in rat blood and bile using a microdialysis sampling technique coupled with HPLC. In addition, further exploration of the mechanism concerning the hepatobiliary excretion of ranitidine is also observed by comparing the pharmacokinetics of ranitidine present both with and without cyclosporine, a *P*-gp inhibitor, and quinidine, an OCT inhibitor, subsequently.

2. Experimental

2.1. Chemicals and reagents

Ranitidine was purchased from Aldrich (Milwaukee, WI, USA). Cyclosporine (Sandimmun) and quinidine were obtained from Novartis Pharma (Basle, Switzerland) and Sigma (St. Louis, MO, USA), respectively. Triply deionized water from Millipore (Bedford, MA, USA) was used for all preparations.

2.2. Animal experimentation

All experimental protocols involving animals were reviewed and approved by the institutional animal experimentation committee of the National Research Institute of Chinese Medicine. Male specific pathogen-free Sprague–Dawley rats weighing 250–300 g were obtained from the Laboratory Animal Center of the National Yang-Ming University, Taipei, Taiwan. Following arrival, the animals were kept in our animal facilities for acclimatization for about 7 days, during which they had free access to food (Laboratory Rodent Diet 5P14, PMI Feeds, Richmond, IN, USA) and water until 18 h prior to being used in experiments, at which time only food was removed. The rats were initially anesthetized with urethane 1 g/ml and α-chloralose 0.1 g/ml (1 ml/kg, i.p.), and remained anesthetized throughout the experimental period. The femoral vein was exposed for drug administration. The rat's body temperature was maintained at 37 °C with a heating pad during the experiment.

2.3. Liquid chromatography

Liquid chromatographic grade solvents and reagents were obtained from E. Merck (Darmstadt, Germany). The HPLC system consisted of a chromatographic pump (BAS PM-80, West Lafayette, IN, USA), an off-line fraction collector (CMA 140, Stockholm, Sweden) equipped with a 20 μl sample loop, and an ultraviolet detector (Varian, Walnut Creek, CA, USA). Ranitidine in the dialysate was separated using an Agilent, extended reversed-phase C_{18} column (Zorbax, 150 mm × 4.6 mm i.d.; 5 μm; USA) maintained at ambient temperature. Samples were eluted with a mobile phase containing acetonitrile–methanol–tetrahydrofuran–20 mM K_2HPO_4 (pH 7.0) (24:20:10:946, v/v), and the flow rate of the mobile phase was 1 ml/min. The mobile phase was filtered through a Millipore 0.45-μm filter and degassed prior to use. The optimal UV detection for ranitidine was set at a wavelength of 315 nm. Output data from the detector were integrated via an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

2.4. Method validation

For assessment of intra- and inter-assay variabilities, ranitidine was assayed (six replicates) at concentrations of 0.1, 0.5, 1, 5, 10, 50, 100, and 500 μg/ml on the same day and on six sequential days, respectively. The accuracy (%Bias) was calculated from the nominal concentration (C_{nom}) and the mean value of observed concentration (C_{obs}) as follows: Bias (%) = $[(C_{obs} - C_{nom})/(C_{nom})]\cdot 100$. The precision relative standard deviation (R.S.D.) was calculated from the observed concentrations as follows: %R.S.D. = [standard deviation (S.D.)/C_{obs}]·100. Accuracy (%Bias) and precision (%R.S.D.) values of limit of the quantification was pre-defined as within ± 15%.

2.5. Microdialysis experiment

Blood and bile microdialysis systems consisted of a CMA/100 microinjection pump (CMA, Stockholm, Sweden) and microdialysis probes. The dialysis probes for blood (1 cm in length) [8] were made of silica capillary in a concentric design with the tips covered by dialysis membrane (Spectrum, 150 μm outer diameter with a cut-off at nominal molecular mass of 13 000, Laguna Hills, CA, USA). The blood microdialysis probe was positioned within the jugular vein/right atrium (toward the heart) and then perfused with anticoagulant citrate dextrose, ACD solution (citric acid 3.5 mM; sodium citrate 7.5 mM; dextrose 13.6 mM) at a flow-rate of 2.6 μl/min.

The bile duct microdialysis probe was made in our laboratory [9,10]. A 7 cm dialysis membrane was inserted into a polyethylene tubing (PE-60; 0.76 mm i.d., 1.22 mm o.d.; Clay-Adams, NJ, USA). The ends of the dialysis membrane and PE-60 were inserted into a silica tubing (40 μm i.d., 140 μm o.d.; SGE, Australia) and PE-10 (0.28 mm i.d., 0.61 mm o.d.), respectively. Both the ends of tubing and the union were cemented with epoxy and allowed to dry for a period of 24 h. Following bile duct cannulation, the probe was perfused with Ringer's solution at a flow rate of 2.6 μl/min. Bile dialysates were then analyzed by the HPLC system. Both blood and bile microdialysis sampling were carried out on the same animals. All animals were used only once.

The in vivo probe recovery was determined by estimating the loss (the extraction ratio) of the ranitidine, which was calculated from the concentration in the dialysate (C_{out}) relative to the concentration of the ranitidine in the perfusate (C_{in}). Recovery (R_{dial}) was expressed using the following equation: $R_{dial} = 1 - (C_{out}/C_{in})$ [7].

2.6. Pharmacokinetic analysis

The concentrations of ranitidine in rat dialysates were determined from the calibration curves. The midpoint of the 10 min periods was used as the sampling time for the construction of blood and bile ranitidine microdialysate concentration–time profiles. After a 2-h post-surgical stabilization period, ranitidine (10 or 30 mg/kg, i.v.) was administered to the control group ($n = 6$). For cyclosporine or quinidine treatment, 10 mg/kg of cyclosporine or quinidine was administered via the left femoral vein 10 min prior to ranitidine injection. The volume of each injection was 1 ml/kg. Blood and bile dialysates were assayed by liquid chromatography on the same experimental day. Ranitidine concentrations in blood and bile were corrected by the estimated in vivo recoveries from the respective microdialysis probes.

Microdialysate concentrations (C_m) of ranitidine were converted to unbound concentration (C_u) as follows: $C_u = C_m/R_{dial}$. Pharmacokinetic calculations were performed on each individual set of data using the pharmacokinetic calculation software WinNonlin Standard Edition Version 1.1 (Pharsight, Mountain View, CA, USA) by the noncompartmental method. The area under the concentration–time curve (AUC) and the area under the first moment curve (AUMC) were calculated according to the log linear trapezoidal method. The clearance (Cl) and mean residence time (MRT) were calculated as follows: Cl = dose/AUC; MRT = AUMC/AUC.

2.7. Statistics

The results are represented as mean ± standard error of the mean. Statistical analyses were performed with SPSS version 10.0 (SPSS, Chicago, IL, USA). One-way ANOVA was followed by a Dunnett's post-hoc test comparison between the control (ranitidine treated alone), cyclosporine, and quinidine treated groups. All statistical tests were performed at the two-tailed 5% level of significance.

3. Results and discussion

The present validated liquid chromatographic method was coupled to the microdialysis technique and employed to determine ranitidine disposition from rat jugular vein blood and bile following drug administration. The method demonstrated excellent chromatographic selectivity with no endogenous interferences at the peak for ranitidine. Retention time of ranitidine was about 6.5 min. The mobile phase contains 5% acetonitrile and 95% phosphate buffer, ranitidine was not separated well from biological dialysate, because of the higher hydrophilicity was not capable to improve its separation in the matrix (Figs. 1 and 2). Tetrahydrofuran and acetonitrile show strong extraction ratio into the stationary phase in comparison with methanol [11]. In this study, methanol and tetrahydrofuran are used concurrently with acetonitrile and so ranitidine was adequately separated with the highest peak symmetries, particularly at an optimal mobile phase of acetonitrile–methanol–tetrahydrofuran–20 mM K_2HPO_4 (pH 7.0) (24:20:10:946, v/v).

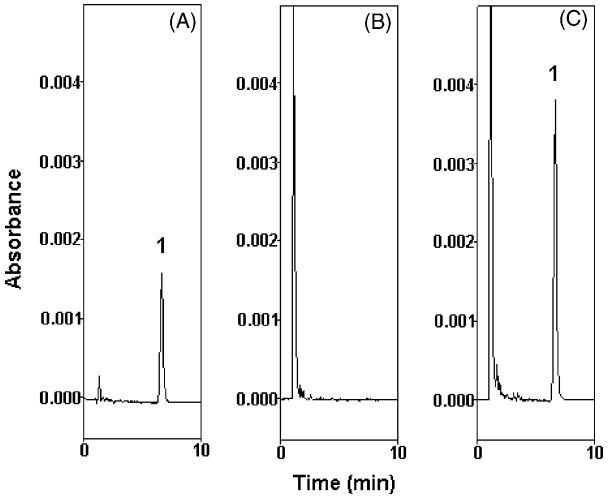

Fig. 1. Typical chromatograms of: (A) standard ranitidine (1.0 μg/ml); (B) blank blood dialysate from the microdialysis probe before drug administration; and (C) blood dialysate sample containing ranitidine (2.0 μg/ml) collected 10–20 min after ranitidine administration (10 mg/kg, i.v.). 1: ranitidine.

Fig. 1A shows a standard injection of ranitidine (1.0 μg/ml), and Fig. 1B the chromatogram of a blank blood dialysate. Fig. 1C shows the chromatogram of a blood

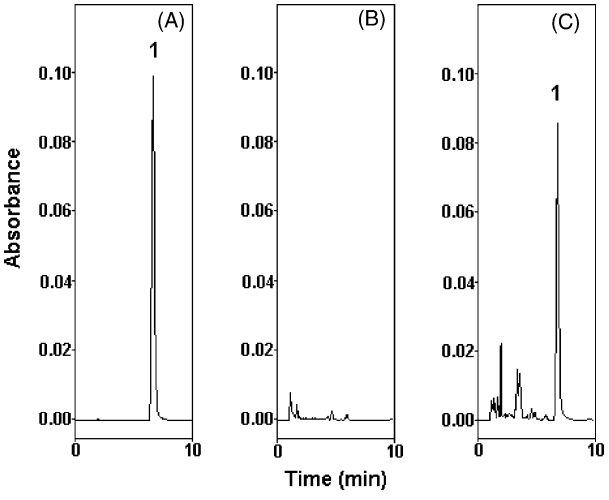

Fig. 2. Typical chromatograms of: (A) a standard ranitidine (50 μg/ml); (B) a blank bile dialysate from the flow-through microdialysis probe before drug administration; and (C) a bile dialysate sample containing ranitidine (43.9 μg/ml) collected 20–30 min after ranitidine administration (10 mg/kg, i.v.). 1: ranitidine.

Table 1
Intra- and inter-assay precision (R.S.D.) and accuracy (Bias) of the HPLC method for the determination of ranitidine

Nominal concentration (μg/ml)	Observed concentration (μg/ml)	R.S.D. (%)	Bias (%)
Inter-assay			
0.1	0.11 ± 0.01	9.1	10.0
0.5	0.48 ± 0.01	2.1	−4.0
1.0	1.01 ± 0.01	1.0	1.0
5.0	4.93 ± 0.15	3.0	−1.4
10	10.1 ± 0.28	2.8	1.0
50	49.9 ± 0.03	0.1	−0.02
100	101 ± 1.72	1.7	1.0
500	499 ± 0.21	0.04	−0.2
Intra-assay			
0.1	0.11 ± 0.01	8.3	10.0
0.5	0.47 ± 0.02	4.3	−6.0
1.0	1.04 ± 0.06	5.8	4.0
5.0	5.08 ± 0.15	3.0	1.6
10	9.98 ± 0.18	1.8	−0.2
50	49.8 ± 0.54	1.1	−0.4
100	101 ± 2.36	2.3	1.0
500	499 ± 0.28	0.06	−0.2

Data expressed as means ± S.D. (n = 6).

dialysate sample containing ranitidine (2.0 μg/ml) collected 10 min after ranitidine administration (10 mg/kg).

Fig. 2 illustrates representative chromatograms of ranitidine in the dialysate of rat bile. Fig. 2A shows the chromatogram of a standard ranitidine (50 μg/ml), and Fig. 2B shows the chromatogram of a blank bile dialysate. None of the observed peaks interfered with the analyte. Fig. 2C shows the chromatogram of bile dialysate sample containing ranitidine (43.9 μg/ml) collected 20–30 min after ranitidine administration (10 mg/kg).

Under the experimental conditions describe above, the limit of detection (LOD) was approximately 0.05 μg/ml, at a signal-to-noise ratio of 3:1. The intra- and inter-assay precision and accuracy of ranitidine were well within the predefined limits of acceptability (15%) (Table 1). The lower limit of quantification (LOQ) was 0.1 μg/ml which was the lowest concentration of analyte. The average microdialysate recoveries of ranitidine for blood (0.5, 1, and 5 μg/ml) and bile (10 and 50 μg/ml) were 25.8 ± 1.3% and 69.6 ± 4.0%, respectively. This method is sufficiently sensitive to allow measurement of ranitidine in rat blood and bile for pharmacokinetic study. Microdialysis membrane excludes large molecules and thus simplifying sample cleaning up procedures

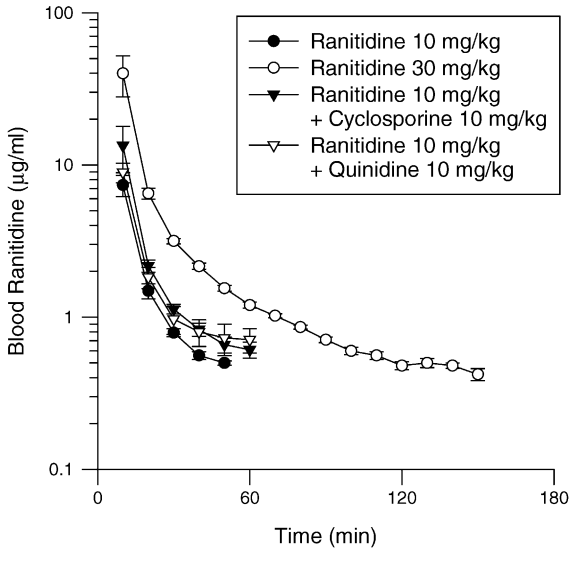

Fig. 3. Concentration–time profiles for ranitidine in blood after ranitidine i.v. administration at dosage of 10 or 30 mg/kg and co-administered with cyclosporine (10 mg/kg) and quinidine (10 mg/kg). Each group of data is represented as means ± S.E.M. from six individual microdialysis experiments.

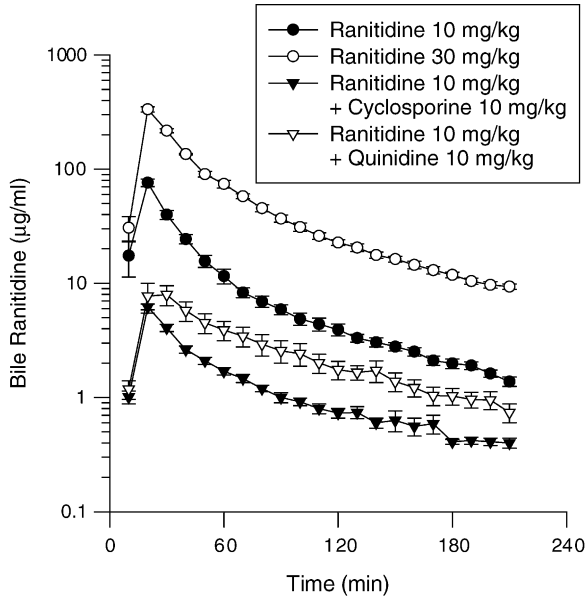

Fig. 4. Concentration–time profiles for ranitidine in bile after ranitidine i.v. administration at dosage of 10 or 30 mg/kg and co-administered with cyclosporine (10 mg/kg) and quinidine (10 mg/kg). Each group of data is represented as means ± S.E.M. from six individual microdialysis experiments.

Table 2
Pharmacokinetic data of ranitidine (10 or 30 mg/kg) in rat blood and bile, both with and without treated with cyclosporine (10 mg/kg) or quinidine (10 mg/kg)

Drug treatment	Ranitidine (10 mg/kg)			Ranitidine (30 mg/kg)
	Alone	With cyclosporine	With quinidine	
Blood				
AUC (min μg/ml)	298 ± 57	780 ± 380	390 ± 64	2140 ± 917
MRT (min)	8 ± 1	13 ± 5	9 ± 1	12 ± 4
Cl (ml/kg/min)	41 ± 8	29 ± 9	30 ± 5	27 ± 87
Bile				
AUC (min μg/ml)	2460 ± 208	321 ± 14[a]	655 ± 118[a]	12700 ± 544
C_{max} (μg/ml)	76.2 ± 5.7	6.2 ± 0.4[a]	8.8 ± 1.9[a]	335 ± 18.2
T_{max} (min)	20 ± 0	20 ± 0	23.3 ± 2.1	20 ± 0
MRT (min)	55 ± 2	84 ± 6[a]	107 ± 9[a]	64 ± 2
AUC_{bile}/AUC_{blood}	9.8 ± 1.9	0.9 ± 0.3[a]	1.8 ± 0.5[a]	13.9 ± 3.8

Data are expressed as mean ± S.E.M. (n = 6).
[a] $P < 0.05$ significantly different from the ranitidine alone (10 mg/kg) group.

for continuous analysis of unbound drugs in various sites of tissues and organs [7]. The dialysates were not needed for further preparation.

Mean ranitidine concentrations in blood and bile versus time curves at the dosages of 10 and 30 mg/kg are presented in Figs. 3 and 4, respectively. These pharmacokinetic profiles suggest that the pharmacokinetics of ranitidine in rat blood and bile exhibited dose-related in the dosage ranges of 10–30 mg/kg. The concentration of ranitidine in the bile was rapidly increased and arrived at a peak concentration in 20–30 min and then follows by a slow declining phase. After drug administration, the concentrations of ranitidine in bile were significantly higher than those in blood, suggesting active excretion of ranitidine from bile-to-blood (Table 2). The hepatobiliary excretion of ranitidine was defined as the bile-to-blood distribution (k value), which was calculated by dividing the ranitidine AUC in bile by that in blood ($k = AUC_{bile}/AUC_{blood}$) [12]. The bile-to-blood distributions were 9.8 ± 1.9 and 13.9 ± 3.8 after ranitidine injection at doses of 10 and 30 mg/kg, respectively.

Cyclosporine (10 mg/kg, i.v.) or quinidine (10 mg/kg, i.v.) co-administration does not alter the blood profile of ranitidine concentrations (Fig. 3) at dosage of 10 mg/kg. However, both cyclosporine and quinidine dramatically decreased the biliary level of ranitidine at dosage of 10 mg/kg (Fig. 4). The bile AUC of ranitidine alone (10 mg/kg), the cyclosporine and quinidine treated group were 2460 ± 208 min μg/ml, 321 ± 14 min μg/ml, and 655 ± 118 min μg/ml, respectively (Table 2). These results suggest that the efflux transport system of ranitidine was inhibited by cyclosporine and quinidine.

The pharmacokinetic profile of healthy volunteers after oral or intravenous administration of the drug presents at least two peaks [13,14]. Some reports have described the existence of these peaks in the plasmatic profile as a result of an enterohepatic recycling process [15–17]. However, Suttle and Brouwer, [18] reported that the secondary peak in the pharmacokinetic curve after oral drug administration is altered by bile flow but not enterohepatic recirculation influences the pharmacokinetics of ranitidine in the rat. Our data provide further information that ranitidine goes through hepatobiliary excretion with active transport.

P-glycoprotein is highly expressed on the bile canalicular membrane of hepatocytes [19]. Many drugs can be excreted by *P*-glycoprotein for the hepatobiliary excretion. The extruded compounds would ultimately leave the body via the feces, which resulted in total clearing and detoxifying from the body. Using Caco-2 monolayer method, ranitidine secretion was significantly reduced by the *P*-glycoprotein substrates of verapamil and cyclosporin [20]. The research indicated that the secretory transport of ranitidine across Caco-2 cell monolayer is mediated by *P*-glycoprotein in the apical membrane [21]. *P*-glycoprotein accelerates hepatobiliary excretion and plays an important role in drug disposition [12]. Our data indicate that the bile-to-blood distribution ratios for ranitidine alone, the cyclosporine and quinidine treated group were 9.8 ± 1.9 min μg/ml, 0.9 ± 0.3 min μg/ml, and 1.8 ± 0.5 min μg/ml, respectively. This result reveals that the hepatobiliary excretion of ranitidine was regulated by *P*-glycoprotein.

In conclusion, we have developed a specific, sensitive, endogenous interference-free microdialysis sampling method for the determination of protein-unbound ranitidine in rat blood and bile. In this paper, we have shown the pharmacokinetic mechanism of unbound ranitidine in blood and bile after intravenous administration to rats.

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